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Serendipita sacchari sp. nov. from a sugarcane rhizosphere in southern China

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Abstract—We isolated a new species, proposed here as *Serendipita sacchari*, from a sugarcane rhizosphere in Guangxi Province, China. This species is characterized by its unstable nucleus numbers (1–15) in its chlamydospores versus their regular distribution in hyphal cells. ITS rDNA and combined LSU+ TEF1-α sequence analyses also support the uniqueness of this new plant symbiont.

Key words—molecular phylogeny, *Sebacinales*, *Serendipitaceae*, taxonomy

Introduction

Serendipita P. Roberts (*Basidiomycota, Sebacinales, Serendipitaceae*), typified with *S. vermifera* (Oberw.) P. Roberts, originally comprised seven species (Roberts 1993). Two additional new species *S. lyrica* Trichiès (Trichiès 2003), and *S. herbamans* K. Riess & al. (Riess & al. 2014) have been proposed in this genus, and two anamorphic species in *Piriformospora* Sav. Verma & al. have been recombined as *S. indica* (Sav. Verma & al.) M. Weiss & al., and *S. williamsii* (Zuccaro & M. Weiss) M. Weiss & al. (Verma & al. 1998; Basiewicz & al. 2012; Weiß & al. 2016). *Serendipita* currently contains 11 species and DNA barcodes are widely accepted as an important tool in delineating species (Schoch & al. 2012; Riess & al. 2014).

[#] Ling Xie & Yan-Yan Long contributed equally to this work.

During an investigation of plant endophytes in sugarcane rhizosphere in Guangxi Province, China, an interesting strain has been morphologically and phylogenetically confirmed as a new species of *Serendipita* and is described herein.

Materials & methods

Soil samples were collected from a 5–15-cm sugarcane rhizosphere layer in Tanluo Town, Nanning City, Guangxi Province, China, in Feb 2011. Fungal isolates were obtained by baiting with Chinese cabbage as described by Narisawa & al. (1998), cultured on PDA media, and deposited in the China General Microbiological Culture Collection Center (CGMCC3.19906). A pure culture of the fungus was air-dried and frozen to prepare the holotype specimen, which is curated in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Observations and measurements were made with an Olympus BX53 Ci-L light microscope and Tescan-vega3 LMU scanning electron microscope (SEM).

DNA extraction, amplification, sequencing

Genomic DNA was extracted using the Sangon Biotech Rapid Fungi Genomic DNA Isolation Kit from mycelia grown at 28 °C for 10 d on oatmeal medium (OA, 10 g oatmeal, 10 g bacto agar, 1 g $MgSO_4 \bullet 7H_2O$, 1.5 g H_2PO_4, 1 g $NaNO_3$, for 1 L distilled water). The internal transcribed spacer of ribosomal DNA (ITS rDNA), large subunit of ribosomal DNA (LSU), and translation elongation factor 1-α (TEF1-α) were amplified with fungal specific primer pairs ITS1/ITS4, LROR/LR5, and EF1-983f/EF1-2218r, respectively (Vilgalys & Hester 1990, Basiewicz & al. 2012). PCR reaction mixture and conditions followed the protocol of 2×EasyTaq PCR SuperMix. The DNA was amplified in 50 µL volumes containing PCR buffer [20 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 20 mM Tris–HCl, pH8.4], 200 µM of each dNTP, 15 pmol of each primer, 100 ng DNA template, and 2.5 units of Taq DNA polymerase (Biocolor BioScience & Technology). Thermal cycling began with 5 min of denaturation at 94 °C, followed by a 35 cycles of denaturation (94 °C for 40 s) + annealing (56 °C for 40 s) + extension (72°C for 60 s), and finalized with a final extension at 72 °C for 10 min. A negative control using sterilized distilled water instead of template DNA was included in the amplification process. The PCR products were examined by electrophoresis at 75 V for 2 h in 0.8 % (W/V) agarose gel in 1×TAE buffer (0.4 M Tris, 50 mM NaOAc, 10 mM EDTA, pH 7.8) and visualized under an ultraviolet light after staining with ethidium bromide (0.5 µg mL^{-1}). The PCR products were purified using MultiScreen® PCRµ96 Cleanup Filter Plates according to the manufacturer's protocol. Purified PCR products were directly sequenced with primer pairs mentioned above in an ABI 3730-XL DNA sequencer. The sequences were deposited at GenBank (http://www.ncbi.nlm.nih.gov) and compared through a BLAST search.

Table 1. Taxa with GenBank ITS accession numbers

Species	Voucher	GenBank no.
Cantharellus avellaneus	1217/ER	KX857081
Chaetospermum camelliae	MFLUCC12-0433	KF516965
C. chaetosporum	CBS:154.59[T]	KJ710461
Craterocolla cerasi	TUB 020203	KF061265
Ditangium altaicum	LE 231836 [T]	NR 163760
Efibulobasidium albescens	—	AF384860
Globulisebacina rolleyi	—	AY509550
Helvellosebacina concrescens	TUB 019706	JQ665516
H. helvelloides	TUB 019983	KF000415
Paulisebacina allantoidea	—	AF490396
	RoKi 179	KF061266
*Sebacina candid*a	TUB 020331	KF061278
S. pallida	TUB 019650	JQ665562
Serendipita herbamans	S1[T]	KF061285
S. indica	DSM 11827[T]	KF061284
S. sacchari	**CGMCC3.19906 [T]**	**KY496808**
S. williamsii	DAR 29830	KY509323
Tremellodendron ocreatum	TH8577	KT339265
Tremelloscypha dichroa	VB4212	KF061281

Table 2. Taxa with GenBank LSU and TEF1-α accession numbers

Species	Voucher	GenBank no.	
		LSU	TEF1-α
Sebacina incrustans	TU110840	FJ644513	KF313907
Serendipita. indica	DSMZ 11827[T]	AY505557	AJ249911
S. sacchari	**CGMCC3.19906 [T]**	**KY496809**	**MF196313**
S. vermifera	MAFF 305835	DQ983814	JN211111
	MAFF 305838	DQ983816	JN211116
	MAFF 305828	DQ520096	JN211115
S. williamsii	DAR 29830	AY505556	JN211110

Phylogenetic analyses

The three DNA loci were separately aligned using Clustal-X (1.83). Two neighbour-joining trees were derived from ITS1-5.8S-ITS2 and the combined LSU+ TEF1-α datasets using MEGA v. 4 (Tamura & al. 2007)(Tables 1,2; Figs 2,3).

Bayesian analyses of the aligned DNA sequence dataset were conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) following Sun & Guo (2010). The best-fit evolutionary model was determined for each dataset by comparing different evolutionary models via MrModeltest v. 2.3 (Nylander 2008). Four simultaneous Markov Chain Monte Carlo chains were run by starting from random trees and sampling every 100 generations. The analyses were halted at 4,000,000 generations after the calculation reached stationarity. Of the 40,000 trees generated, 25% were

excluded as "burn in" when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the remaining 50% majority rule consensus trees. Significant support was defined by clades contained in >95% of the sampled trees.

Taxonomy

Serendipita sacchari L. Xie, Y.Y. Long & Y.L. Chen, **sp. nov**. Fig. 1

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Differs from other *Serendipita* species by its variable nucleus numbers (1–15 nuclei) in the chlamydospore cells.

Type: China. Guangxi Province: Nanning City, Tanluo Town, in sugarcane rhizosphere, 22°55′30″N 107°58′34″E, alt. 96 m, Apr. 2011 (**holotype**, HMAS 247074; ex-type culture CGMCC3.19906; GenBank KY496808, KY496809, MF196313).

Etymology: *sacchari*, referring to the plant from whose rhizosphere the species was first collected.

Colonies reaching 50 mm in diam on PDA medium at 28 °C after 2 weeks, light yellow, circular (or nearly circular), less aerial hyphae. Hyphae hyaline to light yellow-green, septate, 1.2–3.4 μm (2.2 ± 0.5 μm, n = 50), each cell regularly containing 1–2 nuclei. Chlamydospores abundant, spherical to pear-shaped, singly on top of hypha, aseptate. Spores 11.1–17.6 μm (13.5 ± 1.3 μm, n = 55) in diameter, variably containing 1–15 nuclei. Neither conidiophores nor sexual structures observed.

Sequences & the phylogenetic analyses

The obtained ITS (610 bp), LSU (945 bp), and TEF1-α (1131 bp) sequences were deposited in GenBank. To determine the phylogenetic position of *Serendipita sacchari,* all available ITS, LSU, and TEF1-α sequences from *Serendipita* and related genera were downloaded from GenBank (Tables 1, 2).

The ITS sequences from one isolate of *Serendipita sacchari*, five *Serendipitaceae*, nine *Sebacinaceae*, three *Sebacinales* incertae sedis, and one outgroup *Cantharellus avellaneus*, were included in the phylogenetic analysis. In the alignment of these 19 sequences, the data matrix comprised 591 characters. The alignment dataset was analyzed using MrBayes, applying the GTR+G model selected by MrModeltest as the best-fit model. The prior probability density is a flat Dirichlet (all values = 278 1.0) with both Revmatpr and Statefreqpr as default settings. Posterior probability (BPP) and bootstrap (BS) values are shown on the branches of the ITS Bayesian tree (Fig. 2). The ITS phylogeny places *S. sacchari* in *Serendipitaceae* and in a clade with *S. indica* (BPP = 0.83; NJ support = 97%).

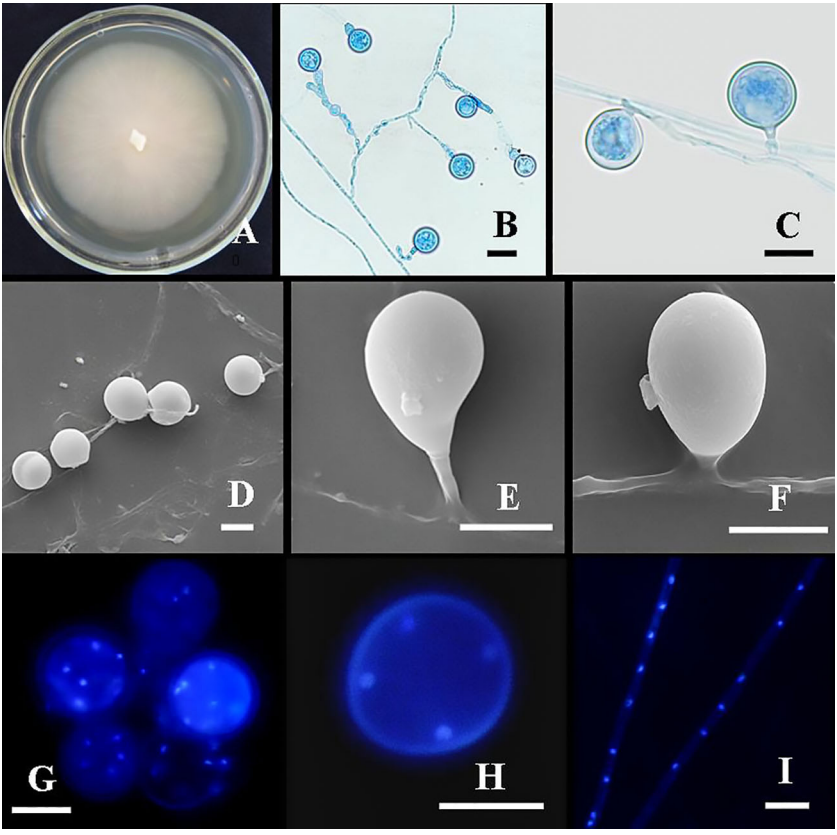

Fig. 1. *Serendipita sacchari* (ex-type, CGMCC3.19906). A: Colony; B, C: Bright field microscopic image of chlamydospores; D–F: Scanning electron microscopic image of chlamydospores; G, H: DAPI–stained chlamydospores; I: DAPI-stained hyphae. Scale bars: B–I = 10 μm.

A combined LSU+TEF1-α dataset comprising one *Serendipita sacchari* isolate, five *Serendipita* isolates, and the outgroup *Sebacina incrustans* was included in a second phylogenetic analysis. These seven sequence pairs (LSU+TEF1-α) aligned in a 1990-character data matrix. The alignment dataset was analyzed in MrBayes, applying the GTR+G model selected by MrModeltest as the best-fit model. The prior probability density is a flat Dirichlet (all values = 278 1.0) with both Revmatpr and Statefreqpr as default settings. Here also BPP and BS values are shown on the branches of the combined Bayesian tree (Fig. 3).

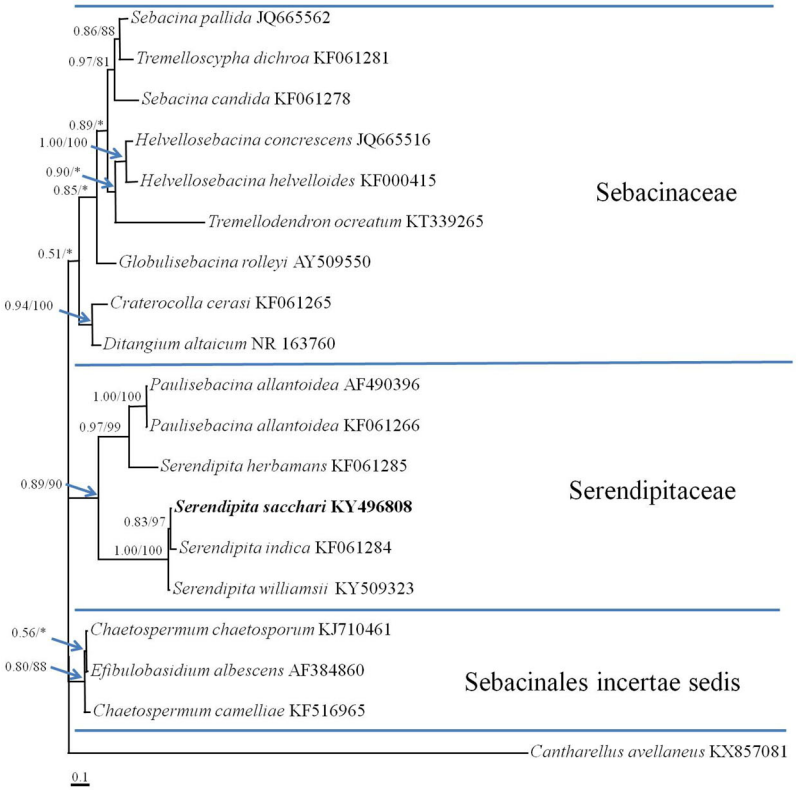

Fig. 2. Phylogenetic tree based on ITS sequences of *Serendipita* and related *Sebacinales* species. *Cantharellus avellaneus* was included as outgroup. The numbers at each branch represented Bayesian posterior probabilities (left) and bootstrap support calculated from 1000 replicates (right). The sequence derived from the proposed new species is in bold. Bar = 0.1 expected changes per site.

In the LSU+TEF1-α phylotree, *S. sacchari* formed a clade with *S. williamsii* with 0.95 BPP support and 100% NJ support. A comparison of the ITS, LSU and, TEF1-α sequence dataset indicates that *S. sacchari* differs from *S. williamsii* in 12/530 bp (2.3%, ITS), 15/930 bp (1.6%, LSU), and 60/1068 bp (5.6%, TEF1-α); and from *S. indica* in 22/552 bp (4.0%, ITS), 21/938 bp (2.2%, LSU), and 63/1132 bp (5.6%, TEF1-α).

Comments. Four species in *Serendipita*—*S. herbamans*, *S. indica*, *S. williamsii*, and *S. vermifera*—were closely related to and allied with *S. sacchari*. Neither conidiophores nor sexual structures were observed in these four taxa. *Serendipita sacchari* can be easily distinguished from *S. herbamans*

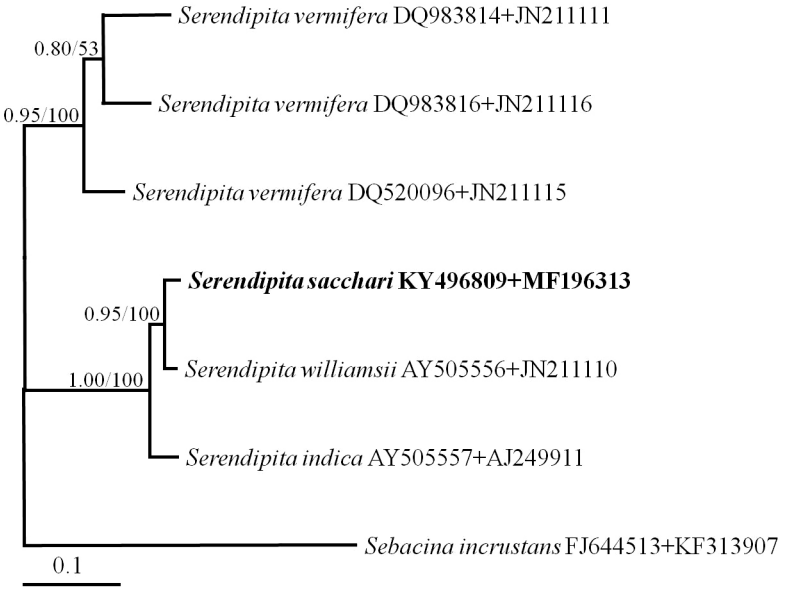

Fig. 3. Phylogenetic tree based on the combined LSU+TEF1-α sequence data of *Serendipita* species. *Sebacina incrustans* was designated as outgroup. The numbers at each branch point represented Bayesian posterior probabilities (left) and bootstrap support calculated from 1000 replicates (right). The sequence derived from the proposed new species is in bold. Bar = 0.1 expected changes per site.

and *S. vermifera* by its bigger (11.1–17.6 µm diam.) chlamydospores (3–5 µm × 4–5 µm in *S. herbamans*; approximately 8 µm diam. in *S. vermifera*; Riess & al. 2014, Ray & Craven 2016). The main difference among *S. sacchari*, *S. indica*, and *S. williamsii* is the distribution and number of nuclei in the cells (Basiewicz & al. 2012). Nuclear number in the chlamydospores of *Serendipita sacchari* (1–15 nuclei) distinguishes the new species from *S. indica* (8–25 nuclei) and *S. williamsii* (≤10 nuclei) (Basiewicz & al. 2012). Moreover, hyphal cells of *S. sacchari* have fewer nuclei and exhibit a more regular distribution compared with *S. williamsii* (2–6 nuclei irregularly distributed) (Verma & al. 1998; Basiewicz & al. 2012). Morphological traits and phylogenetic data both support the recognition of *Serendipita sacchari* as an independent species.

Serendipita herbamans is a common species in plant roots and widely distributed across agricultural and grassland ecosystems. It has the potential to promote the growth of herbaceous plants (Riess & al. 2014). *Serendipita*

indica (≡ *Piriformospora indica*) is widely distributed and well known as a symptomless root endophyte that colonizes bryophytes, pteridophytes, gymnosperms and angiosperms. It has been reported to occur in four continents, is extremely versatile in its mycorrhizal associations, and is known for its ability to promote plant growth (Varma & al. 2012). *Serendipita williamsii* (≡ *Piriformospora williamsii*) was isolated from the spore of arbuscular mycorrhizal fungal in Australia (Basiewicz & al. 2012). *Serendipita vermifera* was isolated from the Australian orchid *Cyrtostylis reniformis* (Warcup 1988). It is reported to induce beneficial effects on plant performance including growth promotion, increase nutrient uptake, enhance seed production, and increase resistance against different biotic and abiotic stresses (Ray & Craven 2016). *Serendipita sacchari* was from sugarcane rhizosphere soil, and symbiotic with Chinese cabbage and sugarcane as a symptomless root endophyte (data not shown). All five *Serendipita* species are plant symbiotic microbionts.

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